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Effects of Arsenite on DNA Repair in *Escherichia coli*

by Toby G. Rossman,* M. Stephen Meyn,† and Walter Troll*

Since environmental exposure to arsenicals has been correlated with a high skin cancer risk among populations exposed to sunlight, it is possible that arsenicals might interfere with the repair of damage to DNA (mostly thymine dimers) resulting from the ultraviolet rays in sunlight. To test this hypothesis, strains of *E. coli*, differing from each other only in one or more repair functions, were exposed to UV light and then plated in the presence or absence of sodium arsenite. Survival after irradiation of wild type *E. coli* (WP_2) was significantly decreased by 0.5*mM* arsenite. This effect was also seen in strains which are unable to carry out excision repair, suggesting that arsenite inhibits one or more steps in the post-replication repair pathways. This is confirmed by the finding that arsenite has no effect on the post-irradiation survival of a recA mutant, which does not carry out post-replication repair.

Mutagenesis after ultraviolet irradiation depends on the rec^+ and lex^+ genes. Arsenite decreases mutagenesis in strains containing these genes. In order to determine its mechanism of action, dose-response relationships of arsenite on a number of cellular functions were carried out. The most sensitive cellular functions found were the induction of β-galactosidase and the synthesis of RNA. Since error-prone repair in *E. coli* is an inducible process, the inhibition of mutagenesis after UV irradiation may be the result of inhibition of messenger RNA synthesis.

Introduction

Progress in the last decade has lead to the finding that most (if not all) chemical carcinogens or their metabolic products are able to combine with DNA (*1*, *2*). The recent development of a microbial mutagenesis assay system has resulted in the finding of a good correlation between mutagenicity in Salmonella and carcinogenicity in animals (*3*). The mutagenicity of many metals was tested by Nishioka using *E. coli* (*4*). Toxic levels of arsenite were found to be mutagenic in *E. coli* capable of postreplication repair.

Epidemiological evidence suggests a correlation between arsenicals and cancer, a relationship which has not been supported by laboratory experiments (*5*). However, an agent might act as a cocarcinogen if it affects the repair of damage to DNA by other agents. This mechanism of action has been proposed for tumor promoters (*6*). Cocarcinogens, according to this model, might turn normal cells into phenocopies of Xeroderma pigmentosum cells, which carry a genetic defect for DNA repair leading to multiple skin cancers (*7*). Workers in Germany have reported that arsenic compounds may enhance the ultraviolet sensitivity of human epidermal fibroblasts (*8*). This suggested to us that arsenicals may act as cocarcinogens by inhibiting some aspect of DNA repair. Since DNA repair is best understood in *E. coli*, and since a variety of repair-deficient mutants exist, we decided to test this hypothesis on *E. coli*, using ultraviolet irradiation as our agent for DNA damage and assaying for survival after irradiation in the presence and absence of sodium arsenite.

Repair of Ultraviolet-Induced Damage in *E. coli*

The primary cause of death and mutations due to ultraviolet irradiation is the formation of pyrimidine dimers in the DNA of the irradiated cell (*9*). In *E. coli*, dimers can be repaired by a variety of path-

*Department of Environmental Medicine, New York University Medical Center, New York, New York 10016.
†Department of Pathology, New York University Medical Center, New York, New York 10016.

ways. We shall be concerned only with "dark repair," i.e., repair that does not involve photoreactivation. Dark repair pathways are illustrated in Figure 1. The major excision repair pathways (the top two pathways in Fig. 1) involve an endonuclease attack on the DNA near the pyrimidine dimer, followed by exonuclease action to remove a section of DNA which includes the dimer itself. This is followed by replacing the missing nucleotides by polymerase action, using the remaining undamaged strand of DNA as a template, and rejoining the newly synthesized region to the original strand by ligase action. In *E. coli*, the major branches of excision repair are error-free (i.e., nonmutagenic) (*10, 11*). If all dimers are repaired by these pathways, no mutations will arise from ultraviolet irradiation. A minor component of excision repair (inducible, error-prone repair) resembles the SOS repair described below.

If all dimers have not been repaired by the time DNA replication takes place, the replicating fork will not proceed past the dimers. DNA synthesis halts at or near a dimer and reinitiates at a later point, resulting in daughter strand gaps of a fairly large size. The repair of these gaps depends on the *recA* gene. The branches of this pathway are not well understood. It is now thought that the major repair is recombinational, constitutive, and error-free. The repair of most interest to those concerned with mutagenesis is the SOS repair, labeled "Inducible, Error Prone" in Figure 1. For a review of this type of repair, see Witkin (*12*). Error-prone repair activity responsible for mutagenesis in *E. coli* is repressed in undamaged cells. In response to damage to DNA, induction of a number of functions takes place, among which are induction of λ phage and SOS repair. Strains of *E. coli* lacking a functional *recA* or *lex* (exr) gene are nonmutable by UV light.

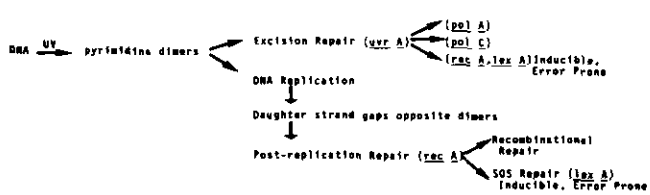

FIGURE 1. Dark repair pathways in *E. coli*, and the genes needed for various branches of these pathways.

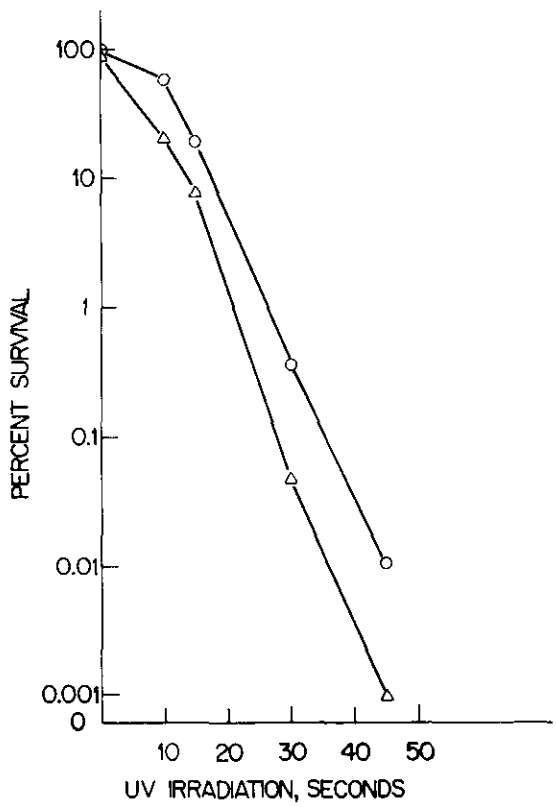

FIGURE 2. Ultraviolet survival curves of strain WP_2 plated in the absence (o) or presence (△) of 1*mM* sodium arsenite.

Effect of Arsenite on Ultraviolet-Irradiated *E. coli*

We have obtained from Dr. Evelyn Witkin strains of *E. coli* which differ from each other only in repair capacity. The effect of sodium arsenite on postirradiation survival was determined for each of these strains by methods which have been described previously (*13*). A typical survival curve of a strain which has full repair capacity is shown in Figure 2. The effect of including 1*mM* sodium arsenite in the plating medium is to eliminate most of the shoulder and to slightly increase the slope of the curve. Thus it appears as if arsenite inhibits DNA repair, since 1*mM* arsenite has no effect on the viability of unirradiated bacteria.

In order to determine the branch(es) of repair which are affected by arsenite, survival curves were obtained for the strains deficient in various repair pathways. The results are summarized in Table 1. Since arsenite decreases survival of ultraviolet irradiated *E. coli* which are deficient in excision repair (WWP_2 and WP_6), while having no effect on strains which cannot carry out any postreplication repair (WP_{10}), it follows that arsenite inhibits postreplication repair, most likely by inhibiting a *recA*-dependent function. The increased survival of WP_5 by arsenite may be explained as follows: arsenite

Table 1. Effects of arsenite on survival after ultraviolet irradiation.

Strain	Relevent phenotype	Survival after 186 erg/mm² UV, %	Effect of 1 *mM* Arsenite on survival
WP_2	wild type	59.4	decrease
WWP_2	$UvrA^-$	0.4	decrease
WP_5	$ExrA^-$ ($LexA^-$)	0.02	increase
WP_6	$PolA^-$	15.0	decrease
WP_{10}	$RecA^-$	0.0004	no effect

might inhibit the excessive DNA degradation which normally occurs in *lex*⁻ and *recA*⁻ strains. The *lex*⁻ strain would then be able to perform recombinational repair resulting in increases survival. This hypothesis is under investigation.

The effects of various concentrations of sodium arsenite on the viable count (colony-forming ability) of irradiated and unirradiated bacteria is shown on Figure 3. Concentrations of arsenite up to 5*mM* have no effect on the viable count of unirradiated *E. coli*, although at 1*mM* and higher, the colony size is smaller, indicating inhibition of growth rate. Concentrations of arsenite of 0.1*mM* and higher decrease the survival of irradiated wild-type cells and increase the survival of irradiated WP_5.

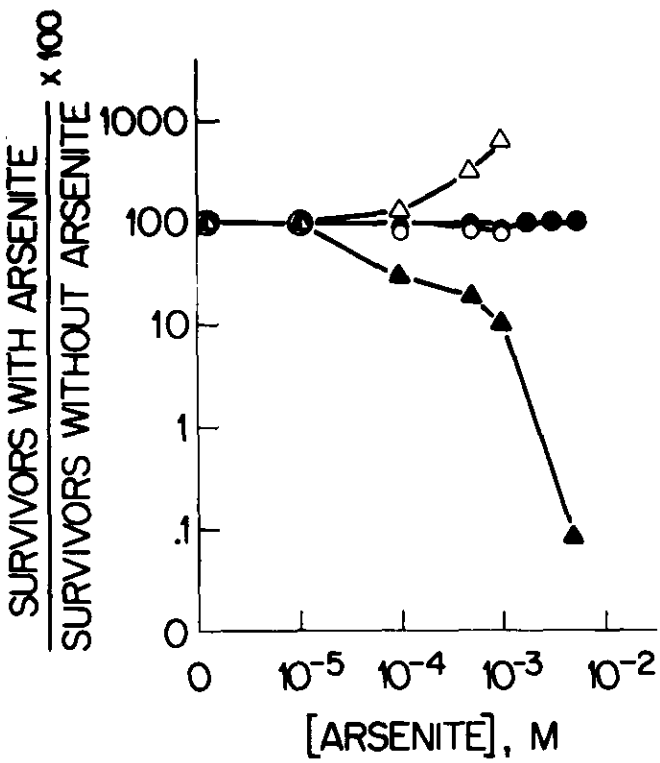

FIGURE 3. Effect of arsenite concentration on the survival of unirradiated and irradiated cultures. (●) WP_2 unirradiated; (▲) WP_2, irradiated to 0.01% survival; (○) WP_5, unirradiated; (△) WP_5, irradiated to 0.02% survival.

Mutagenesis after ultraviolet irradiation is due to SOS repair. If arsenite inhibits *recA*-dependent functions, it should reduce the number of mutations after ultraviolet irradiation, since SOS repair requires a functional *recA* gene. Using strain WWP_2, which lacks excision repair and therefore is more easily mutable by ultraviolet light than is wild type, we measured the mutation frequency after exposure to ultraviolet light using the method of Witkin to score for *trp*⁺ revertants *(14)*. The results are shown in Table 2. Arsenite decreases the absolute mutation frequency and the number of mutations per survivor at all ultraviolet exposures. The effect is not a very large one, however, when compared with an agent such as the protease inhibitor antipain, which can reduce ultraviolet mutagenesis by more than 95% *(15)*.

Dose Dependency of Arsenite in the Inhibition of Various Cell Functions

The effect of arsenite on the survival of ultraviolet irradiated *E. coli* increases with increasing irradiation (Fig. 2, Table 3). This is in line with our hypothesis that arsenite affects post-replication repair, which becomes more important at higher UV doses due to saturation of excision repair capacity *(16)*. Also supporting our hypothesis is the finding that the arsenite effect is dependent upon cell growth. If irradiated bacteria are held in buffer (in which no cell growth takes place) containing arsenite for 2–6 hr after irradiation, and then plated on medium without arsenite, there is no decrease in survival compared with control cells held in buffer without arsenite. If, however, irradiated cells are held in liquid growth medium containing arsenite

Table 2. Effects of arsenite on ultraviolet × induced mutagenesis.

Ultraviolet, erg/mm²	Arsenite concn, *mM*	Survival, %	Number of Trp⁺ revertants: Per 10^7 bacteria plated	Number of Trp⁺ revertants: Per survivor
0	0	100	4.4	4.4×10^{-7}
0	1	100	4.2	4.2×10^{-7}
12.5	0	100	95.7	95.7×10^{-7}
12.5	1	100	69.3	69.3×10^{-7}
50	0	21.2	136.0	644×10^{-7}
50	1	17.1	70.6	411×10^{-7}
100	0	0.869	46.5	5350×10^{-7}
100	1	0.650	21.5	3240×10^{-7}

for 2 hr and then plated without arsenite, the arsenite effect occurs. This indicates that arsenite must be present under conditions of cell growth in order to decrease post-irradiation survival.

Table 3. Concentrations of arsenite inhibiting cell functions 50%.

Function	Medium	ID_{50}, *mM*
Survival after 50 erg/mm² UV	Glucose-SEM agar	1.6
Survival after 75 erg/mm² UV	Nutrient agar	1.1
Survival after 300 erg/mm² UV	Nutrient agar	0.07
Mutation frequency after 50 erg/mm² UV	Glucose-SEM agar	1.6
Growth rate	Nutrient broth	1.0
Growth rate	Minimal A + glucose + tryptophan	1.6
Growth rate	Minimal A + glycerol + tryptophan	0.48
Induction of β-galactosidase	Minimal A + glucose + tryptophan	0.36
Induction of β-galactosidase	Minimal A + glycerol + tryptophan	0.13
RNA synthesis	Minimal A + glucose + tryptophan	0.36
RNA snythesis	Minimal A + glycerol + tryptophan	0.10
Protein synthesis	Minimal A + glycerol + tryptophan	0.55

Although arsenite must be present under conditions of growth in order to decrease survival after ultraviolet irradiation, arsenite itself slows down growth at concentrations similar to those needed for decreased postirradiation survival. The magnitude of arsenite's growth inhibitory effect depends upon the medium used. Growth in a minimal medium with glycerol as the carbon source is especially sensitive to arsenite (Table 3).

Since many *recA*-dependent functions are now thought to be inducible (*12*), it follows that inhibition of RNA synthesis, protein synthesis or induction mechanisms (such as removal of repressors from DNA) by arsenite would result in inhibition of *recA*-dependent functions. Using methods described previously (*17*), we performed a series of dose-response experiments on the effects of arsenite on RNA synthesis, protein synthesis, and the induction of β-galactosidase (an operon unrelated to DNA repair). The concentrations of arsenite inhibiting these functions 50% are presented in Table 3.

The most sensitive functions to arsenite inhibition were the induction of β-galactosidase and RNA synthesis. Protein synthesis was slightly less sensitive. Since enzyme induction requires the synthesis of new messenger RNA, the inhibition of β-galactosidase induction might be a secondary effect, resulting from the inhibition of RNA synthesis. As was the case for growth rate, the sensitivity of *E. coli* to arsenite inhibition of RNA synthesis and β-galactosidase induction varied in different growth media, greater sensitivity being found when glycerol was the carbon source than when glucose was the carbon source.

Conclusions

We have shown that arsenite inhibits DNA repair in *E. coli*, and that this effect is specific for postreplication repair. The mechanism of action is not clear, partly because postreplication repair pathways are not well understood. However, it does appear that arsenite does not specifically inhibit SOS repair because of the small effect on ultraviolet-induced mutation frequency. The effect on SOS repair (which is inducible) might be a result of inhibition by arsenite of RNA synthesis. We also have preliminary evidence that degradation of DNA after irradiation may be inhibited by arsenite.

Interference with DNA repair is a possible mechanism for cocarcinogenesis. We are now planning to investigate the effects of arsenite on DNA repair in mammalian cells in culture. Mammalian DNA repair differs from that found in *E. coli*. For example, postreplicative gaps in animal cells are filled by *de novo* DNA synthesis rather than by the recombination mechanism which predominates in *E. coli* (*18*). Agents therefore need not affect repair the same way in the two systems. Caffeine, which inhibits excision repair in *E. coli*, has been shown to inhibit postreplication repair in mammalian cells (*19*).

It is likely that any interference with DNA repair in mammalian cells could lead to increased carcinogenesis. Xeroderma pigmentosum cells have various defects in DNA repair (*20*), yet the clinical symptoms are similar. Pyrimidine dimers caused by sunlight lead to skin cancer whether the genetic defect leads to decreased excision repair or defective postreplfcation repair.

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